# Genetic diversity of the VP7, VP4 and VP6 genes of Korean porcine group C rotaviruses

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**Abstract**

Porcine group C rotaviruses (RVCs) are considered important pathogens due to their economic impact on pig industry and may also cross the host species barrier toward humans. Unlike RVA, however, genetic and phylogenetic data on RVCs from pigs and other host species are scarce. In the present study, full-length ORF sequences of 26 VP7, 9 VP4 and 9 VP6 genes of Korean porcine RVC strains were compared with those of other known RVC strains by phylogenetic analyses and pairwise identity frequency graphs. Applying the established 85% nucleotide identity cut-off value for RVC VP7 classification, the 26 Korean porcine RVC strains belonged to the G1, G3, G6 and G7 genotypes. Although more complete RVC VP4 sequences are warranted before a definitive cut-off value could be determined, a provisional 83% nucleotide cut-off value proposed for RVC VP4 classification resulted in 7 P-genotypes, 5 of which possessed porcine RVC strains. A 90% nucleotide cut-off value for VP6 divided RVC strains into 7 I-genotypes, 5 of which had porcine RVC strains. G/P/I-genotype comparisons suggested the occurrence of rather frequent reassortment events among Korean porcine RVC strains, and strong geographical differences in the distribution of RVC G-genotypes worldwide. Our data indicate that a large genetic diversity exists among porcine

RVC strains. For the final genotype determination of each gene segment, more intensified epidemiological studies on animal and human RVC strains throughout the world ~~is~~ are needed.

**Keywords:** Porcine; Group C rotavirus; VP7; VP4; VP6; Genetic diversity

# 1 Introduction

Rotavirus (RV), a member of the *Reoviridae* family, is one of the major pathogens that causes severe and acute dehydrating diarrhea in young children and in a wide variety of domestic animals (Desselberger, 2014). The RV genome is enclosed in three concentric capsid layers and is comprised of 11 segments of double-stranded (ds) RNA, encoding six structural proteins (VP1-4, VP6 and VP7) and five or six nonstructural proteins (NSP1-NSP5/6) (Desselberger, 2014). A viral capsid protein VP6, located between the core capsid VP2 and the two outer capsid proteins VP4 and VP7, exposes group-specific antigens (Desselberger, 2014). Based on VP6 antigenic properties and sequence diversity, RVs are further classified into 7 groups (RVA–RVG) (Desselberger, 2014). Recently, the human RV strains NADRV-J19, ADRV-N and NADRV-B219 were tentatively assigned to a novel RV species H (RVH) (Matthijnssens et al., 2012).

RVC was first detected in pigs in 1980 (Saif et al., 1980), and has been subsequently identified in humans (Rodger et al., 1982), ferrets (Torres-Median, 1987), cattle (Tsunemitsu et al., 1991) and dogs (Otto et al., 1999). Like RVAs, human RVCs are also known as global pathogens able to cause severe gastroenteritis worldwide (Esona et al., 2008). Porcine RVCs are widespread in nursing, weaning, and post-weaning pigs with diarrhea either alone or in mixed infection with other enteric pathogens (Amimo et al., 2013; Martella et al., 2007a; Marthaler et al., 2013). In addition, porcine RVC infections have been associated with symptoms ranging from asymptomatic infection to clinical infections leading to severe diarrhea in either sporadic episodes or large outbreaks (Collins et al., 2008; Jeong et al., 2009). However, thorough molecular analysis of porcine RVCs has only been carried out in the United States (Amimo et al., 2013; Marthaler et al., 2013; Tsunemitsu et al., 1996), Italy (Martella et al., 2007a~~b~~,b), Ireland (Collins et al., 2008), South Korea (Jeong et al., 2009), Canada (Marthaler et al., 2013), and the Czech Republic (Moutelíková et al., 2014).

The zoonotic potential of animal RVCs has been postulated based on increased sero-prevalence rates to RVCs in human populations living in rural settings (Iturriza-Gómara et al., 2004) and analyzing archival fecal samples of Brazilian children (Gabbay et al., 2008). Interspecies transmission of RVCs between different animal species was identified in which bovine strain WD534tc is believed to have originated from pigs (Chang et al., 1999). In addition, the VP6 gene detected in the piglet belonged to the bovine RVC lineage, indicating possible interspecies transmission or genetic reassortment of RVC between bovine and porcine RVCs (Jeong et al., 2009).

Unlike RVAs, the molecular characterization of RVC strains has been hampered by its fastidious propagation in cell culture and the lack of genomic sequence data on RVCs, hindering the establishment of a proper classification system for RVC strains. Recently a sequence-based classification has been proposed and adopted by the Rotavirus Classification Working Group (RCWG) for VP7, defining 9 RVC G genotypes (G1~~–~~G9) (Marthaler et al., 2013). There is a limited number of RVC VP4 and VP6 sequences available, but no official RVC VP4 (P-genotypes) or VP6 (I-genotypes) classification has been proposed (Mawatari et al., 2014; Moutelíková et al., 2014).

In our previous reports using RT-nested PCR and real-time PCR targeting the VP6 gene of RVCs (Chun et al., 2010; Jeong et al., 2009), porcine RVC infections were found to be widespread in Korean piglets with diarrhea. However, the VP7, VP4 or VP6 genotypes of the infecting Korean porcine RVCs remained largely unknown. This prompts us to investigate the VP7, VP4 and VP6 genes of Korean porcine RVCs. In this study, we demonstrate the co-circulation of several porcine RVC G-genotypes, and a high diversity of VP4 and VP6 genes present in RVC strains circulating in Korea, resulting in tentatively novel P and I genotypes. The findings from our study provide important information on the evolution and genetic diversity of circulating RVC strains.

# 2 Materials and methods

## 2.1 Specimens

A total of 88 fecal specimens from nursing, weaning, post-weaning, grower, finish pigs and sows with or without diarrhea housed on 27 farms from 4 provinces were selected from archived fecal samples which were collected between 2004 and 2012 (Table 1). All porcine fecal samples used in this study were found to be positive by conventional one-step RT-PCR and/or nested PCR with RVC VP6-specific primer pairs (Supplementary Table 1) and were sequenced for confirmation (data not shown).

**Table 1** Information of fecal samples from which full-length ORF sequences of VP7, VP4 and VP6 genes of RVCs were obtained.

| Fecal ~~N~~no. | County[a] | Year of collection | Age[b] | Diarrhea status[c] | Genotypes[d] | | |
|---|---|---|---|---|---|---|---|
| | | | | | G | P | I |
| 04-155-1 | G | 2004 | 42 | D | 3 | | |
| 04-155-5 | G | 2004 | 60 | D | 1 | | 7 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 04-97-1 | I | 2004 | 60 | D | 7 | | |
| 04-105-2 | Go | 2004 | 56 | D | | | 7 |
| 06-52-1 | M | 2006 | 35 | D | | | 6 |
| 06-52-2 | M | 2006 | 150 | D | 7 | | |
| 06-69-1 | M | 2006 | 30 | D | 7 | | |
| 06-92-1 | Y | 2006 | 6 | D | | | 5 |
| 06-144-2 | S | 2006 | 70 | D | 3 | 1 | 7 |
| 06-176-1 | C | 2006 | 42 | D | 6 | | |
| 06-238-2 | Y | 2006 | 35 | D | 1 | | |
| 06-268-2 | J | 2006 | 63 | D | | 5 | |
| 06-281-4 | M | 2006 | 15 | D | 6 | | |
| 07-60-4 | Gi | 2007 | 80 | D | 6 | 5 | |
| 07-74-11 | M | 2007 | 35 | D | 1 | 7 | 4 |
| 07-109-12 | S | 2007 | 6 | D | 6 | 4 | |
| 08-128-1 | C | 2008 | 5 | D | 7 | | 4 |
| 08-148-2 | Go | 2008 | 3 | D | | 7 | |
| 09-15-7 | Ye | 2009 | 7 | D | 6 | | 4 |
| 09-15-9 | Ye | 2009 | 8 | D | 3 | | 4 |
| 09-43-8 | Y | 2009 | 5 | D | 3 | | |
| 09-47 | D | 2009 | 21 | D | 3 | | |
| 09-84-5 | Ys | 2009 | 21 | D | 7 | | |
| 11-58-4 | Y | 2011 | Sow | ND | 7 | | |
| 11-58-7 | Y | 2011 | Sow | ND | 7 | | |
| 2885 | Y | 2012 | 22 | ND | 7 | 6 | |
| 1027 | Y | 2012 | 19 | D | 7 | 6 | |
| 2846 | Go | 2012 | 25 | ND | 3 | | |
| 2455 | Go | 2012 | 25 | ND | 3 | | |
| 50-12 | Go | 2012 | 30 | D | 3 | | |
| 61-12 | Y | 2012 | 21 | ND | 6 | | |
| 2478 | Y | 2012 | 55 | D | | 7 | |

[a] G: Gwangyang, I: Iksan, Go: Gochang, M: Muan, S: Shinan, C: Chonan, Y: Yeonggwang, J: Jeongeup, Gi: Gimje, S: Suncheon, Ye: Yeongam, D: Damyang, and Ys: Yesan counties.

[b] The age is in days.

[c] D: Diarrhea, ND: none diarrhea.

[d] Genotypes: Genotypes of VP7, VP4 and VP6 identified in this study are written for each sample.

## 2.2 RNA extraction

The RNA was extracted from a 200 µl starting volume of centrifuged 10% fecal suspensions using the Trizol-LS (Gibco-BRL, Life Tech, Grand Island, NY) procedure. The total RNA recovered was suspended in 50 µl of RNase free water and stored at −80 °C until further use.

## 2.3 RT-PCR

To obtain the full-length ORF sequences of the VP7, VP4 and VP6 genes, primer sets for each gene (Supplementary Table 1) were used as described previously (Amimo et al., 2013; Rahman et al., 2005). RT-PCR assays with different primer sets for detection and sequencing of the VP7, VP4 and VP6 genes of RVCs were performed using a standard one-step RT-PCR as previously described (Jeong et al., 2009). Nested PCR assays with primer pairs specific for VP8* or VP5* were carried out using the RT-PCR products (Jeong et al., 2009). The RT-PCR and nested PCR products were analyzed by 1 or 1.5% agarose gel electrophoresis and visualized by UV after ethidium bromide staining.

## 2.4 DNA sequencing

RT-PCR or nested products amplified by primer pairs specific to each genomic segment were purified using a QIAEX II Gel Extraction kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The extracted PCR products were ligated into the pGEM-T Easy Vector Systems (Promega), and were sub-cloned into home-made XL1-Blue competent cells. Individual colonies were grown and plasmid was purified using Hybrid-Q™ Plasmid (GeneAll, Seoul, South Korea). DNA sequencing was carried out using an ABI System 3700 automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

## 2.5 Genebank accession numbers

The GenBank accession numbers of Korean RVC strains characterized in this study are as follows: KJ814484: KJ814484–KJ814509 for the VP7 genes, KJ814468–KJ814476 for the VP4 genes, and FJ494690–FJ494691 and KJ814477–KAJ814483 for the VP6 genes. Details of Korean and other known strains are shown in Supplementary Table 2.

## 2.6 Phylogenetic analysis

Phylogenetic and molecular evolutionary analyses were conducted at the nucleotide level using MEGA, Version 6.06, software (Tamura et al., 2013). Genetic distances were calculated using the Kimura-2 correction parameter at the nucleotide level, and trees were constructed using the Neighbor joining algorithm.

## 2.7 Construction of pairwise identity graphs

To determine cut-off values for sequence-based classification of the VP7, VP4 and VP6 genomic segments, the percentages of nucleotide identities between the complete ORF sequences of the VP4, VP6 and VP7 gene segments from this study and published sequences available in GenBank (Supplementary Table 2) were calculated using the pairwise distances program of the MEGA program, Version 6.06 (Tamura et al., 2013). To extend sequence based genotyping of VP6 genes, the partial VP6 sequences (1200 bp nucleotides from nt 82 to nt 1281) of nine Czech RVC strains were also analyzed. Pairwise identity frequency graphs were constructed by plotting all the calculated pairwise identities on the X-axis and plotting the frequencies of the calculated pairwise identities on the Y-axis (Ball, 2005).

# 3 Results

## 3.1 Sequence and phylogenetic analyses of the VP7 genes of Korean RVC strains

VP7 genes of RVCs have been divided into 9 G-genotypes based on nucleotide-based pairwise identity frequency graphs and phylogenetic dendrograms (Marthaler et al., 2013). These 9 G-genotypes were based on an 85% cut-off value. In this study, a total of 26 Korean porcine VP7 genes were sequenced and their complete ORF nucleotide sequences were compared with 107 other VP7 sequences of known RVC strains (85 porcine, 9 bovine and 13 human) available from GenBank. By the comparison of the VP7 full-length ORF nucleotide sequences of Korean RVC strains with the representative strains of each G genotype using this 85% cut-off value, the 26 Korean strains were found to belong to the established G1 (3 strains), G3 (8 strains), G6 (6 strains) or G7 (9 strains) RVC VP7 genotypes. Using these full-length ORF nucleotide sequences of the VP7 genes (999 to 1011 bp long), phylogenetic dendrogram was constructed on the nucleotide level (Fig. 1). Phylogenetic dendrogram confirmed that the 26 Korean porcine RVC strains were classified appropriately to the G1, G3, G6 and G7 genotypes, suggesting a large genetic diversity of porcine RVC strains circulating in Korea (Fig. 1).

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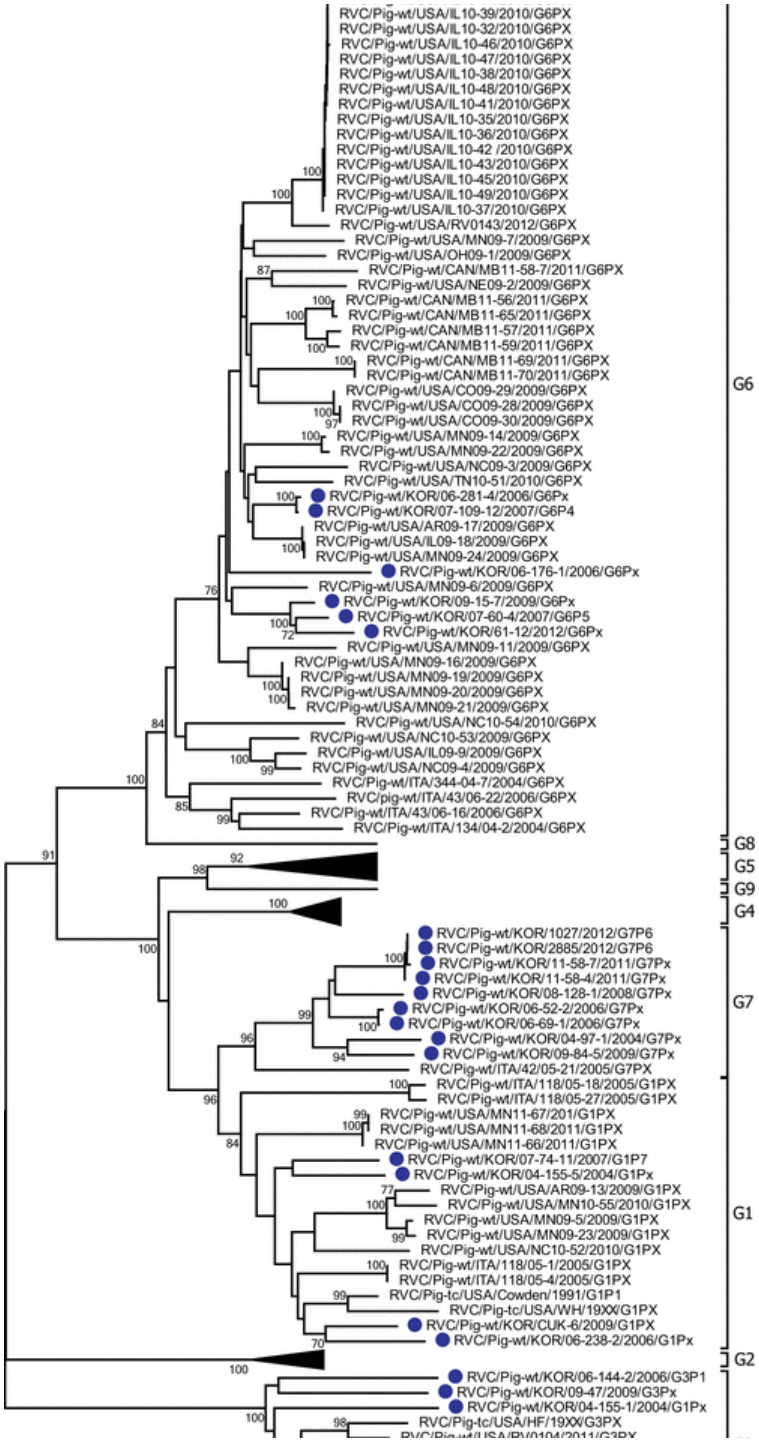

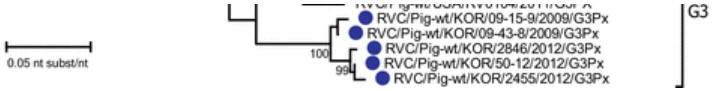


**Fig. 1** Phylogenetic dendrogram of RVC VP7 genes at the nucleotide level. The full-length ORF sequences of VP7 genes of RVC strains are analyzed. Bootstrap values (1000 replicates) above 70 are shown. Certain clusters are replaced by triangles, in which the height of the triangle represents the number of sequences, and the width represents the genetic diversity inside that cluster. Korean porcine strains obtained in this study are indicated with a filled circle.

## 3.2 Sequence and phylogenetic analyses of the VP4 genes of Korean RVC strains

The complete ORF nucleotide sequences (2211 bp from nt 6 to nt 2216) of 9 Korean RVC strains from pigs of various ages (Table 1) were compared with those of 25 other known RVC strains available from GenBank (2 porcine, 8 bovine and 15 human). The phylogenetic dendrogram based on the nucleotide sequences of these VP4 genes showed that porcine, human and bovine RVC strains belonged to distinct clusters/genotypes (Fig. 2A). For classification purposes, several alternative ways to divide the tree into distinct genotypes were investigated together with their respective pairwise identity frequency graphs. Although the analyses were only based on 34 sequences, an 83% nucleotide cut-off value was found to be most suited defining 7 P-genotypes (Fig. 2A and B, including the previously proposed genotypes P1, P2 and P3), each containing RVC strains from a single host species (porcine, human or bovine, respectively). The analyses showed only a single inter-genotype identity below the proposed 83% cut-off value (82% between strains RVC/Pig-wt/USA/RV0143/2013/G6P5 and RVC/Pig-wt/KOR/06-268-2/2006/GXP5 inside genotype P5) and two intra-genotype identities above the proposed 83% cut-off value (85% for strain RVC/Pig-wt/KOR/07-109-12/2007/G6P4 from genotype P4 and strain RVC/Pig-wt/USA/Cowden/1980/G1P1 from genotype P1; 84% for strain RVC/Pig-wt/KOR/2478/2012/GXP7 from genotype P7 and strains RVC/Pig-wt/KOR/06-144-2/2006/G3P1 from genotype P1).

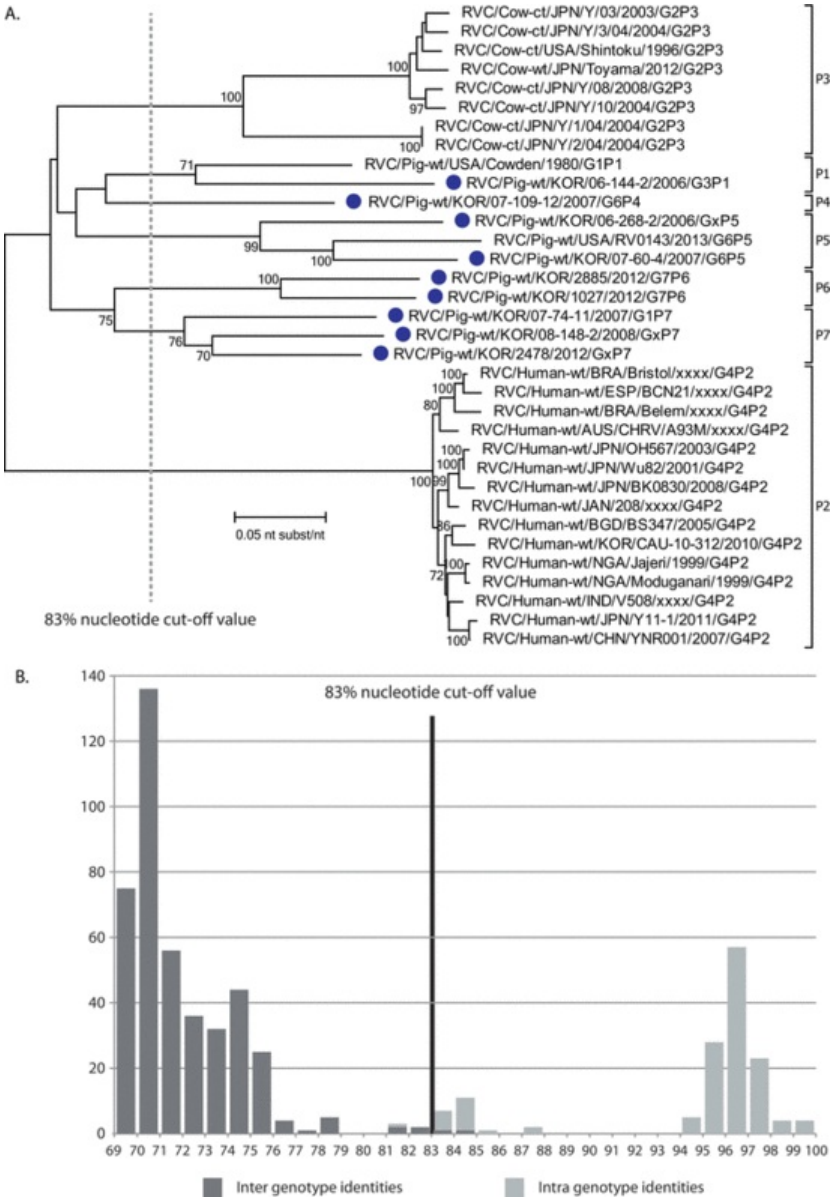


**Fig. 2** (A) Phylogenetic dendrogram of RVC VP4 genes at the nucleotide level. The full-length ORF sequences of VP4 genes of RVC strains are analyzed. Bootstrap values (1000 replicates) above 70 are shown, and porcine strains obtained in this study are indicated with a filled circle. The proposed 83% nucleotide cut-off value is represented by the vertical dashed line. (B) The pairwise nucleotide identity frequency graph of the VP4 ORFs, together with the proposed 83% nucleotide cut-off value, is represented by the vertical line. ~~Y~~ y axis indicates frequency of different identities. Black and gray bars indicate inter-genotype and intra-genotype identities, respectively.

## 3.3 Sequence and phylogenetic analyses of the VP6 genes of Korean RVC strains

The complete VP6 ORF nucleotide sequences (1185 bp from nt 22 to nt 1206) of 9 Korean RVC strains from pigs of various ages (Table 1) were compared with those of 28 other known RVCs available from GenBank (6 porcine, 9 bovine and 13

human). A phylogenetic dendrogram constructed with these 37 VP6 nucleotide sequences is shown in Fig. 3A. As shown in Fig. 3B, the identity frequency graph showed a clear separation into two peaks, suggesting that a 90% cut-off value at the nucleotide level would be appropriate. This cut-off in combination with the phylogenetic dendrogram resulted in a division of the tree into seven I-genotypes. Human and bovine RVC strains made up genotypes I2 and I3, respectively, whereas the porcine RVC strains could be further divided into 5 I genotypes: I1 and I4-I7 (Fig. 3A).

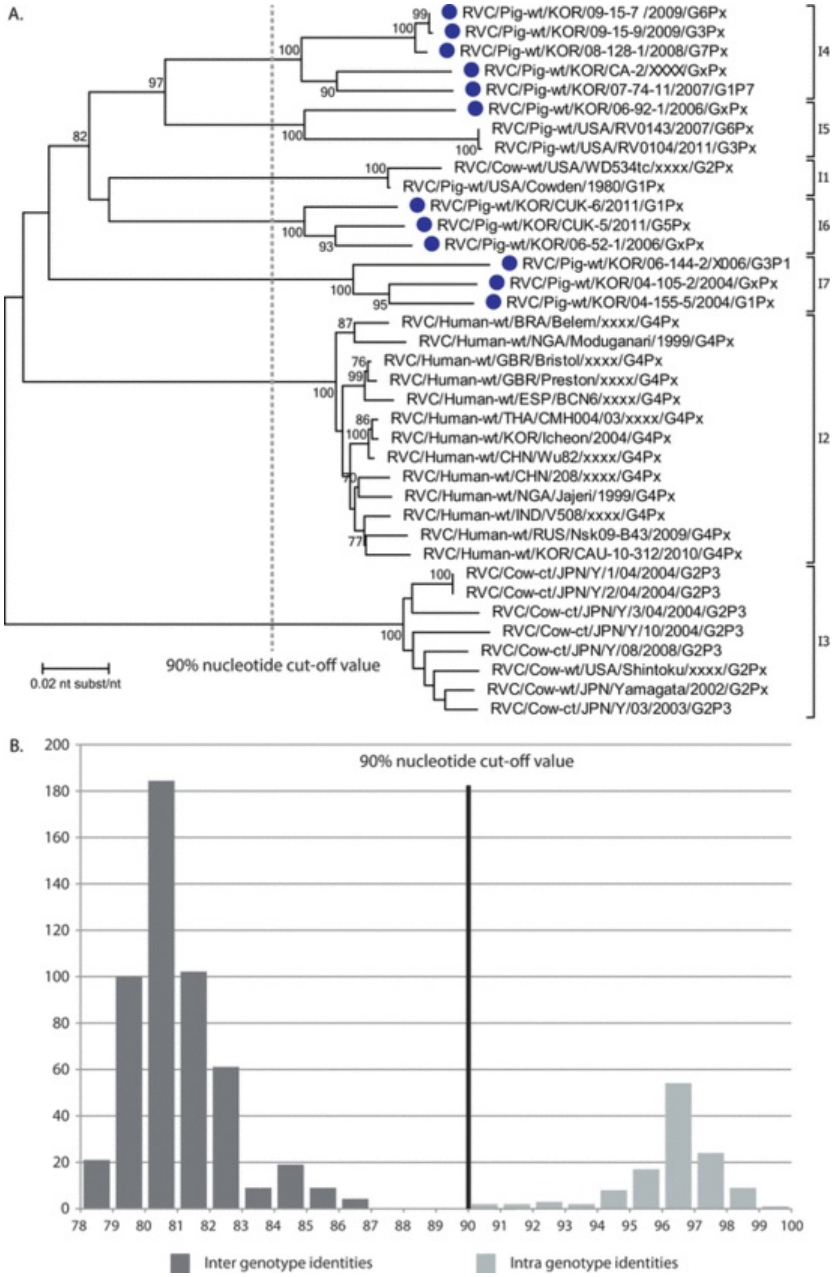


**Fig. 3** (A) Phylogenetic dendrogram of the VP6 genes at the nucleotide level. The full-length ORF sequences of VP6 genes of RVC strains are analyzed, and porcine strains obtained in this study are indicated with a filled circle. Bootstrap values (1000 replicates) above 70 are shown. The proposed

90% nucleotide cut-off value is represented by the vertical dashed line. (B) The pairwise nucleotide identity frequency graph of the VP6 ORFs, together with the proposed 90% nucleotide cut-off value, is represented by the vertical line. Y axis indicates frequency of different identities. Black and gray bars indicate inter-genotype and intra-genotype identities, respectively.

In this analysis, the VP6 sequences of nine Czech RVC strains were not included as their ORF sequences were not completely available (60 nucleotides were missing on the 5′ end of the ORF) (Moutelíková et al., 2014). To further investigate their molecular relationship with our proposed genotypes, 9 Czech RVC strains were aligned with the previously analyzed strains, overlapping sequences (1200 bp nucleotides from nt 82 to nt 1281) were selected and a phylogenetic tree was constructed as can be seen in Fig. S1. This analysis showed that two Czech strains clustered into genotype I4 together with RVC strains from Korea, the USA and Brazil, whereas the other 7 Czech strains formed distinct clusters which could be new genotype according to the proposed 90% cut-off value (data not shown). However, their nucleotide sequences should be completed before they can be ratified as new genotypes.

## 3.4 Reassortment events

Although the number of RVC strains from which more than 1 genotype has been determined is relatively small, Table 1 suggests the rather frequent occurrence of reassortments among porcine RVC strains. Most striking was the observation that the four Korean I4-genotype bearing porcine RVC strains all had different VP7-G genotypes, G1, G3, G6 and G7 (Supplementary Table 3). The VP6 gene nucleotide sequences of strains 09-15-9, 09-15-7 and 08-128-1 were very similar, ranging from 99.2 to 99.9% similarity. The two Korean porcine strains 04-155-5 and 06-144-2 both possess the I7 genotype (93.4% nucleotide sequence identities), but possessed different G genotypes, G1 and G3 (Supplementary Table 3). Furthermore, the two Korean G6-genotype carrying strains 07-60-4 and 07-109-12 (90.7% nucleotide sequence identities) contained the P5 and P4 genotypes, respectively (Supplementary Table 3). These data indicated that reassortment events might occur rather frequently among Korean porcine RVC strains.

# 4 Discussion

Based on phylogenetic analyses and pairwise identity frequency graphs of the VP7 gene of RVC strains, an 85% nucleotide cut-off value has been reported, resulting in the identification of 9 RVC G genotypes (Marthaler et al., 2013). Among these G genotypes, bovine and human RVC strains are genetically conserved and restricted to genotypes G2 and G4, respectively. However, porcine RVC strains are genetically very diverse, and are present in the remaining 7 genotypes (G1, G3, and G5-G9) (Marthaler et al., 2013). The Korean porcine RVC strains from this study were shown to fall into 4 of these porcine G genotypes: G1, G3, G6 and G7. Epidemiological studies conducted on American, Italian, Canadian, and Irish pig farms have demonstrated that three G genotypes (G6, G5 and G1 in decreasing order) are the most frequently detected VP7 genotypes associated with porcine RVC infections (Amimo et al., 2013; Collins et al., 2008; Martella et al., 2007b). The other porcine G genotypes (G3, G7, G8 and G9) formed only a minority of the detected porcine RVC VP7 genotypes. In the present study, however, the two most prevalent G genotypes were G7 and G3, which are extremely rare in other countries, with only one G7 and 2 G3 strains reported from Italia and the USA, respectively (Martella et al., 2007b; Marthaler et al., 2013; Tsunemitsu et al., 1996). In addition, the RVC G6 and G1 genotypes, which are the most popular genotypes in USA and Italia, were the third and fourth most prevalent genotypes in Korea. These results indicate that significant geographical differences exist in the prevalence of different VP7 RVC G genotypes. In this respect, further longitudinal surveillance studies will be crucial to investigate the dynamics of different RVC G-genotypes, and to see if strong genotype fluctuation can occur from one year to the next as is known for RVA.

In pigs, only two complete and one partial nucleotide sequences of RVC VP4 genes are reported from the USA (Amimo et al., 2013; Bremont et al., 1992). By the comparison of these limited porcine RVC VP4 gene sequence data with other known RVC strains sequences from human and bovine, it was shown that both porcine strains Cowden and RV0143 are phylogenetically in separate branches, and share only 74.6% nucleotide sequence identity among each other and below 74.1% nucleotide sequence identities with bovine and human RVC strains (Amimo et al., 2013). Therefore, both porcine RVC strains were proposed to be representative of distinct VP4 genotypes (Amimo et al., 2013). These observations were confirmed in the present study where we analyzed and compared 9 complete VP4 ORF sequences of porcine RVC VP4 genes, with other available VP4 RVC sequences in GenBank. The identify frequency graph along with a phylogenetic dendrogram revealed that an 83% nucleotide cut-off value was found to be most suited, resulting in the identification of 7 distinct P-genotypes in which porcine RVC strains formed 5 P-genotypes (P1, and P4-P7), in addition to the P2 and P3 genotypes, which exclusively contained human and bovine RVC strains, respectively. Several hundred full-length sequences of RVA VP4 gene segments from various host species and countries are available (Matthijnssens and Van Ranst, 2012), which have been classified into 37 distinct P-genotypes (Trojnar et al., 2013). The proposed cut-off value and genotypes for VP4 of RVC are only based on 34 available complete ORF nucleotide sequences, and therefore, more intensified epidemiological studies throughout the world will need to be conducted in order to determine if the currently proposed 83% cut-off value is the most suited for RVC VP4 classification into P-genotypes.

A recent study conducted in the Czech Republic investigated the partial VP6 sequence of porcine RVC strains (Moutelíková et al., 2014). Their phylogenetic analyses suggested that in addition to the human and bovine I-genotype I2 and I3 respectively, the porcine RVC strains formed a large genetically very diverse group (I1), which was further subdivided into 2 different lineages (Moutelíková et al., 2014). Our study adds further support to the large genetic diversity of porcine RVC strains, and in addition phylogenetic dendrogram and pairwise identity frequency graph were constructed with the full-length ORF nucleotide sequences of the RVC VP6 genes, to propose a rational classification into I-genotypes. A 90% nucleotide cut-off value resulted in 7 I-genotypes where human and bovine RVC strains belonged to genotypes I2 and I3, respectively, and porcine RVC strains were classified into genotypes I1 and I4-I7. Our analyses did not include the 9 Czech porcine strains because their sequences lacked the 5′ end ORF sequences. After inclusion of the Czech strains and analyses on the available data, it seems as if the Czech strains might be representatives of at least two novel I-genotypes. As is the case for RVA, novel genotypes should preferentially be determined based on complete ORF sequences, as genetic diversity might not be evenly distributed across the ORFs (Matthijnssens and Van Ranst, 2012). Therefore, these potentially novel genotypes were not yet assigned an I-genotype number, until the complete ORF has been analyzed.

In the present study, no VP7, VP4 and VP6 genotypes typical for RVC strains from other species (human and bovine) were found in the fecal samples obtained from Korean pigs with various ages, not providing evidence on interspecies transmissions. Our analyses based on a limited number of porcine RVC strains revealed the frequent occurrence of reassortment events among these Korean porcine RVC strains in nature. Reassortment of RVA genomic segments, one of the key evolutionary mechanisms of RVAs, might occur in the event of a double infection with two different strains of RVAs in a single host, producing a series of completely novel combinations of genome segments in the progeny viruses, which may give rise to more transmissible or more pathogenic viruses (Desselberger, 2014). In contrast to reassortment events of RVAs which have long been well known (Desselberger, 2014), this study reports for the first time possible reassortment events in RVCs in nature. To understand insight into how RVCs evolve in nature, further in-depth epidemiological studies should globally be carried out with many more samples from porcine as well as humans and bovines.

The RT-PCR and nested-PCR assay with primer pairs targeting the partial sequence of RVC VP6 gene (Gabbay et al., 2008) detected 88 RVC-positive fecal samples in this study. However, RT-PCR and/or nested PCR assays with previously published primer pairs specific for the full-length ORF sequences of the VP7, VP4 and VP6 genes only allowed us to amplify 26 VP7, 9 VP4 and 9 VP6 genes from thirty-two selected fecal samples (Amimo et al., 2013; Rahman et al., 2005). This is most likely a sensitivity issue, resulting from the fact that the initial assay only targeted a small region of VP6 (<356 bp), whereas the latter primers targeted much larger regions of VP7 (1043 bp), VP4 (VP8*, 1222 bp; VP5*, 1179 bp) and VP6 (1352 bp) genes of RVC.

Although the amount of sequence data is still rather limited for RVC, our results show that Korean porcine RVC strains, and porcine RVC strains in general are genetically much more diverse than human and bovine RVC strains, suggesting that pigs are the main reservoir for RVC strains. Further epidemiological studies are likely to reveal the presence of additional genotypes in humans and animals worldwide.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vetmic.2014.12.024.

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# Appendix A. Supplementary data

The following are the supplementary data to this article:

Multimedia Component 1

Multimedia Component 2

Multimedia Component 3

Multimedia Component 4

**Highlights**

- Genetic diversity of porcine RVC strains.
- Novel genotypes of RVC VP4 and VP6 genes.
- Frequent reassortment events of RVC strains.

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